भारतीय मानक
Indian Standard

IS 17318 : 2020

कोन में और अन्य संबद्ध पैकिंग में हिना (*मेहंदी*) पेस्ट — विशिष्टि

Henna (*MEHENDI*) Paste in Shape of Cone and other Allied Packaging — Specification

ICS 71.100.70

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Cosmetics Sectional Committee, PCD 19

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards after the draft finalized by the Cosmetics Sectional Committee had been approved by the Petroleum, Coal and Related Products Division Council.

Henna (called *MEHENDI* in Hindi), Lawsonia Inermis Linn has been known as natural source of dye having medicinal properties. After dilution with water, henna powder is used as paste for skin decorations, tattooing and hair dying. Upon drying, the paste imparts orange, dark red to very deep red that appears almost black on skin while only typical reddish orange colour is imparted on white hair. In order to offer the convenience of ready to use paste to users, henna paste is supplied in different packaging formats like cones, tubes etc. In order to help the users, henna paste is supplied in the conical packing to facilitate several types of designs on the hands and other parts of body as it has better design maneuverability.

Many commercial henna (*MEHENDI*) paste products available in the market lose their staining power on storage especially ones packed in cones. Destabilized paste of henna cones when used does not give any colouration. To increase the staining power and market value, some products are sold with chemicals like dyes and pigments like *p*-phenylenediamine, basic and acidic dyes etc. which are potential allergens when used on skin. Such chemicals are not permitted in manufacture of mehendi/ henna paste.

The henna (*MEHENDI*) paste in the form of cones are manufactured as micro business activity in small households, by road side vendors and small shops, which make paste in small lots, immediately fill in suitable films and sell on the spot. These cones generally do not have a shelf life more than one week. Therefore, it is specified in **4.3** that for henna (*MEHENDI*) paste with shelf life below one month, the 'use before' declaration needs to be made in the DD/MM/YY format. The *p*H and water quality used in manufacture of henna (*MEHENDI*) paste play an important role in its stability and colour impartation.

For the purpose of deciding whether a particular requirement of this standard is complied with the final value, observed or calculated, expressing the result of a test or analysis shall be rounded off in accordance with IS 2 : 1960 'rules for rounding off numerical values (*revised*)'.

Indian Standard

HENNA (*MEHENDI*) PASTE IN SHAPE OF CONE AND OTHER ALLIED PACKAGING — SPECIFICATION

1 SCOPE

This standard prescribes the requirements and methods of sampling and test for henna (*MEHENDI*) paste in shape of cone and other allied packaging.

2 REFERENCES

The following standards contain provisions, which through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
3958 : 1984	Methods of sampling cosmetics (*first revision*)
4011 : 2018	Methods of test for safety evaluation of cosmetics (*third revision*)
4707	Classification of cosmetic raw materials and adjuncts
(Part 1) : 2017	Colourants (*third revision*)
(Part 2) : 2017	List of raw materials generally not recognized as safe for use in cosmetics (*fourth revision*)
14648 : 2011	Microbiological examination of cosmetics and cosmetic raw materials — Methods of test (*second revision*)

3 REQUIREMENTS

3.1 Description

The henna (*MEHENDI*) paste is a stable paste of henna powder in water which imparts colour stains on skin or hair.

NOTE — Small quantities of essential oils, binding agents, suitable preservatives and other auxiliary excipients necessary to impart cosmetic and functional properties to form a smooth and stable paste may be incorporated.

3.2 Ingredients

3.2.1 Unless specified otherwise, all the raw materials used in the manufacture of henna (*MEHENDI*) paste shall conform to the requirements prescribed in the relevant Indian Standards where such standards exist.

3.2.2 All ingredients of henna (*MEHENDI*) paste shall comply with the provisions of IS 4707 (Part 1) and IS 4707 (Part 2) subject to the provisions of *the Drugs and Cosmetics Act*, 1940 and Rules framed there under.

3.2.3 For safety evaluation of novel ingredients used in formulation of henna (*MEHENDI*) paste, it shall comply to IS 4011.

3.3 The henna (*MEHENDI*) paste shall also comply with the requirements given in Table 1 when tested as prescribed in column 4 and 5 of the Table 1.

4 PACKING AND MARKING

4.1 Packing

The henna (*MEHENDI*) paste shall be packed in suitable containers like films drawn into a cone or any suitable packing to give protection during its shelf life. When packed in containers, the containers shall be properly sealed and have a leak-proof cap or closure. The containers, if necessary, may further be packed in cartons or any other suitable packaging material.

4.2 Storage

The henna (*MEHENDI*) paste containers shall be stored away from heat and direct sunlight.

4.3 Marking

4.3.1 The henna (*MEHENDI*) paste containers and the cartons shall be legibly marked with the following information:

a) Name of the material;
b) Name and address of manufacturer;
c) Net content in the container;
d) Batch or lot number in code or otherwise;
e) Month and year of manufacture;

f) List the ingredients (at the time of manufacture) under the title 'ingredients' as follows:
   1) For ingredients more than 1 percent (by mass or volume) — List the ingredients in decreasing order of percentage.
   2) For ingredients less than 1 percent (by mass or volume) — List the ingredients in any order.

   NOTE — This is exempted in case of pack sizes less than 30 g of solid/semi-solid and 60 ml of liquid. The list can be given at outer/inner label or leaflets and/or neck tag securely tied with the package/container depending upon on the specific packing under which it will be sold to the consumer.

g) 'Use before ....' (month and year from the date of manufacture to be declared by the manufacturer. In case, the shelf life is below one month then declaration has to be made in the DD/MM/YY format);

h) Instructions for use;

   NOTE — These can be given at outer/inner label or leaflets and/or neck tag securely tied with the package/container depending upon on the specific packing under which it will be sold to the consumer.

j) Cautions 'DO NOT USE FOR COLOURING EYELASHES OR EYEBROWS. DO NOT USE ON CUT, IRRITATED OR ABRADED SKIN'; and

k) Any other information required by statutory authorities.

4.3.2 *BIS Certification Marking*

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act*, 2016 and the Rules and Regulations framed thereunder, and the products may be marked with the Standard Mark.

5 SAMPLING

5.1 Representative samples of the henna (*MEHENDI*) paste shall be drawn as prescribed in IS 3958.

5.2 Test for all characteristics shall be carried out on the composite sample.

5.3 The henna (*MEHENDI*) paste shall be taken to have confined to the specification if the composite sample passes all the tests.

6 QUALITY OF REAGENTS

Unless specified otherwise, pure chemicals and distilled water [*see* IS 1070 : 1992 Reagent grade water — Specification (*third revision*)] shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the result of analysis.

Table 1 Requirements for Henna (*MEHENDI*) Paste

(*Clause* 3.3)

Sl No.	Characteristic	Requirement	Method of Test, Ref to	
			Annex	IS
(1)	(2)	(3)	(4)	(5)
i)	*p*H of 5 percent aqueous suspension	3.0 to 6.0	A	—
ii)	Loss on drying, percent, *Max*	80	B	—
iii)	Lawsone content in paste form, percent, *Min*	0.25	C or D[1]	—
iv)	Absence of chemical dyes and pigments	To pass the test	E and F	—
v)	Heavy metals as lead (Pb), parts per million, *Max*	20	G	—
vi)	Arsenic (as As_2O_3), parts per million, *Max*	2	H	—
vii)	Microbial count, CFU/g or CFU/ml[2]:			
	a) Total viable count, *Max*	1000	—	IS 14648
	b) Pseudomonas aeruginosa	Absent	—	IS 14648
	c) Escherichia coli	Absent	—	IS 14648
	d) Staphylococcus aureus	Absent	—	IS 14648
	e) Candida	Absent	—	IS 14648

[1] In case of any dispute with respect to lawsone content, method of test prescribed at Annex C shall be the reference method.

[2] CFU/g = for solids; CFU/ml =for liquids

ANNEX A

[*Table* 1, *Sl No.* (i)]

DETERMINATION OF *p*H

A-1 APPARATUS

A-1.1 *p*H Meter — Preferably equipped with glass electrode.

A-2 PROCEDURE

Weigh accurately about 5 g of henna (*MEHENDI*) paste in 250 ml beaker. Add 100 ml water, stir for 5 min. Determine the *p*H of suspension at 27 ± 2°C using the *p*H meter.

ANNEX B

[*Table* 1, *Sl No.* (ii)]

DETERMINATION OF LOSS ON DRYING

B-1 OUTLINE OF THE METHOD

This method determines loss on drying which is the loss in weight, percent (*w/w*) resulting from water and volatile matter of any kind that can be driven off under specified conditions. The test is carried out on a well-mixed sample of the henna (*MEHENDI*) paste.

B-2 APPARATUS

B-2.1 Drying Oven

B-3 PROCEDURE

B-3.1 Weigh a glass stoppered, shallow weighing bottle that has been previously dried at 105 ± 2°C to constant weight and cool in desiccators. Note down the weight (W_1).

B-3.2 Transfer 1 g of sample to the bottle, cover it and accurately weigh the bottle and the contents. Note down the weight (W_2). Distribute the sample as evenly as practicable by gentle sidewise shaking to a depth not exceeding 10 mm.

B-3.3 Place the loaded bottle in the drying oven/desiccators, remove the stopper and keep it also in the drying oven/desiccators.

B-3.4 Dry the sample at 105 ± 2°C to constant weight. After drying is completed, open the drying chamber, close the bottle promptly and allow it to cool to room temperature (where applicable) in a desiccators before weighing. Weigh the bottle and the contents (W_3).

$$\text{Loss on drying, percent} = \frac{\text{Loss in weight} \times 100}{\text{Weight of sample taken}}$$

$$= \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

ANNEX C

[*Table* 1, *Sl No.* (iii)]

DETERMINATION OF LAWSONE CONTENT BY COLORIMETRY

C-1 OUTLINE OF METHOD

The pigment is extracted and the lawsone content is determined by comparing the observed optical density (measured colorimetrically) with a calibration curve, relating optical density to various concentrates of 2-hydroxy-1,4-napthoquinone.

C-2 APPARATUS

C-2.1 Spectrophotometer or Photoelectric Colorimeter (with a filter of 490 nm)

C-3 REAGENTS

C-3.1 Sodium Bicarbonate Solution — 5 percent (*m/v*).

C-3.2 2-Hydroxy-1,4-napthoquinone

C-4 PROCEDURE

C-4.1 Preparation of Standard Calibration Curve

Prepare 0.1 percent standard stock solution of 2-hydroxy-1,4-napthoquinone ($C_{10}H_6O_3$) by dissolving it in sodium bicarbonate solution [5 percent, (*m/v*)]. Pipette 0 ml (blank), 1 ml, 2 ml, 3 ml, 4 ml and 5 ml of 0.1 percent standard stock solution in 100 ml volumetric flasks and dilute with sodium bicarbonate solution [5 percent, (*m/v*)] up to the mark. Measure the optical density of these solutions having varying concentration from 0.0, 10, 20 to 50 ppm of 2-hydroxy-1,4-napthoquinone, at 490 nm with a spectrophotometer and construct a calibration curve. Do not store the stock solution for more than one month.

C-4.2 Weigh accurately, 2.0 g of the dry henna (*MEHENDI*) paste (or wet paste equivalent to 2 g on dry basis) prepared sample. Transfer it to a 100 ml volumetric flask. Add sodium bicarbonate solution [5 percent, (*m/v*)] and make up the volume to mark. Shake the contents of the flask every half an hour or so for about 8 h. Allow to settle overnight. Thereafter filter the solution through a filter paper and reject the first few milliliters. Take 10 ml of the filtrate in a 25 ml volumetric flask and dilute with sodium bicarbonate solution [5 percent, (*m/v*)] up to the mark. Measure the optical density of this solution with a spectrophotometer at 490 nm.

C-5 CALCULATION

Refer to the calibration curve and determine the percent lawsone content of the sample from the curve.

ANNEX D

[*Table* 1, *Sl No.* (iii)]

DETERMINATION OF LAWSONE CONTENT BY HPLC

D-1 CHROMATOGRAPHIC CONDITIONS

D-1.1 Column — C18 HPLC column with 5 µm particle size, pore size 100 Å, 250 mm × 4.6 mm

D-1.2 Mobile Phase — Methanol : water (50:50, *v/v*) with 1 percent *o*-phosphoric acid.

D-1.3 Wavelength — 286 nm.

D-1.4 Flow Rate — 1 ml/min.

D-1.5 Temperature — Ambient.

D-1.6 Run Time — 15 min (approximately).

D-2 SAMPLE PREPARATION

Weigh 2.0 g of sample. Transfer it to a 100 ml volumetric flask. Add sodium bicarbonate solution [5 percent, (*m/v*)] and make up the volume to mark. Shake the contents of the flask every 30 min or so for about 8 h. Allow to settle overnight, thereafter filter the solution through a filter paper and reject the first few milliliter. Take 10 ml of the filtrate in a 25 ml volumetric flask and dilute with distilled water up to the mark. Use this solution for HPLC.

D-3 STANDARD PREPARATION

Take 0.0213g/100 ml of standard 2-hydroxy-1,4-naphthoquinone and prepared same as described under sample preparation.

D-4 STABILITY AND STORAGE PREPARATION

The standard and sample are stable when stored in glass volumetric flask and are refrigerated.

D-5 CALCULATIONS

Lawsone content, percent (on wet paste basis) =

$$\frac{\text{Area of sample} \times \text{Concentration of standard} \times \text{Purity percent (actual)}}{\text{Area of standard} \times \text{Concentration of sample}}$$

ANNEX E

[*Table* 1, *Sl No.* (iv)]

DETERMINATION OF ABSENCE OF CHEMICAL DYES AND PIGMENTS BY TLC

E-1 OUTLINE OF METHOD

To detect contamination of chemical dyes or synthetic pigments if any, the Thin Layer Chromatography (TLC) method shall be used.

E-2 APPARATUS

E-2.1 Weighing Balance

E-2.2 TLC Plates — Precoated silica gel 60 F 0.2 mm thickness

E-2.3 TLC Apparatus / Beakers — 250 ml (narrow)

E-2.4 Iodine Chamber

E-2.5 Test Tubes, with stoppers

E-2.6 Volumetric Flask, 10 ml

E-2.7 Micro Syringe, 10µl

E-2.8 Silicone Wax

E-3 REAGENTS AND STANDARDS

E-3.1 Chloroform

E-3.2 Methanol

E-3.3 Glacial Acetic Acid

E-3.4 Ethyl Acetate

E-3.5 Iodine Granules

E-3.6 Standards — Pure henna, lawsone pigment, chemical/synthetic dye ingredients[1)] like *p*-phenylenediamine, *p*-aminophenol, *m*-aminophenol, 2-nitro-*p*-phenyleneamine, etc.

E-3.7 Essential Oils — Clove oil, terpineol oil, eucalyptus oil, tea tree oil, lemon oil or any other essential oils declared on the label matter by the manufacturer.

E-3.8 Preservatives — Benzyl alcohol or any other preservative declared on the label matter by the manufacturer.

E-3.9 Test Sample — 1 g of henna paste sample being tested.

E-4 PREPARATION OF SOLUTION

E-4.1 Mobile Phase

Chloroform : ethyl acetate : methanol : glacial acetic acid (8:2:1:1 *v/v*). Take the solvents chloroform, ethyl acetate, methanol and glacial acetic acid in 8:2:1:1 *v/v*, mix well and keep stoppered.

E-4.2 Sample Solutions

Dissolve about 1 g of test sample with chloroform into the 10 ml flask and make up volume to ml with chloroform then centrifuge at 4 000 rpm for about 10 min and use the supernatant liquid for spotting.

E-4.3 Reference Solutions

Weigh about 50 mg ± 1 mg of the reference substance in 10 ml flask. Dissolve and dilute to 10 ml with chloroform. Prepare 0.5 percent (*w/w*) solution of each reference substances lawsone, clove oil, eucalyptus oil and terpineol separately. Additionally, if required, *p*-phenylenediamine, *p*-aminophenol, *m*-aminophenol, 2-nitro-*p*-phenyleneamine, or any other suspected dyes separately.

E-4.4 Preparation of Henna Powder Reference Sample

Disperse about 3 g of henna powder prepared from authentic henna leaves in water and make a smooth paste, allow to stand for 4 h. Mix 1 g of this paste with chloroform into the 10 ml flask and make up volume to 10 ml with chloroform then centrifuge at 4 000 rpm for about 10 min and use the supernatant liquid for spotting and apply one drop of the extract on the base line of the plate

E-5 PROCEDURE

E-5.1 Pour 15-20 ml of mobile phase into the 250 ml narrow beaker (developing chamber) and cover with a suitable petri dish using silicone wax as a sealant.

E-5.2 Apply to a chromatography plate, about 5 µl of each of above described reference solutions and sample solution along a line approximately 1.5 cm from the edge of the plate.

E-5.3 Place the plate in a tank previously saturated with the development solvents and allows to develop

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1) To be used if unknown spots are seen on the sample plates.

at room temperature in the tank until the solvent front moved about 80 percent of plate height.

E-5.4 Remove the TLC plate, allow the plate to dry completely and place a plate in iodine chamber to develop the chromatogram.

E-6 IDENTIFICATION

E-6.1 Compare the R_f value and the color obtained from the sample with those of the reference substances chromatographed and identify spots. Compare the TLC pattern of the sample with that of the lawsone reference sample and standard henna paste Sample using henna powder reference sample which is prepared from authentic henna leaves.

E-6.2 Any other dye spots on the chromatogram other than lawsone, binding agents, essential oils and chlorophyll indicates presence possible extraneous dyes. In case, unknown spots from suspected chemical dyes are observed, further testing is done by applying the standards of suspected synthetic dyes and check for matching $\mathrm{R_f}$ values and spot characteristics.

Representative elution order of above samples with some commonly used synthetic adulterants (lowest R_f to highest R_f) — *p*-aminophenol, *m*-aminophenol, 2-nitro-*p*-phenyleneamine, lawsone, chlorophyll.

E-6.3 Confirmation of doubtful identification may sometimes be achieved by a spiking method, adding the corresponding reference substance solution to the sample extract. Or if sample shows additional spots, confirmation is to be done by scraping the spot and conducting the UV or IR spectrum.

NOTES:

1. Since the henna (*MEHENDI*) is a natural product, TLC pattern may slightly vary depending upon the change in weather conditions, age of plant, season and different agro-climatic locations. Hence, if other spots are observed in addition to the spots due to chlorophyll and lawsone this needs to be reconfirmed with TLC pattern of henna (*MEHENDI*). Confirmatory may be necessary to conclude the presence of extraneous dyes. This may be done by scraping out the respective spot and solubilizing and checking using other analytical methods like Spectrophotometric, HPLC etc.

2. If the concentration of one or more of the substances found in the sample is excessive, dilute the sample extract and repeat the test.

3. A reddish orange spot due to lawsone will elute, and the greenish spot due to chlorophyll will be coincident with liquid front. Other spots due to the addition of essential oils may also be seen. These spots may interfere with the chemical/synthetic dye ingredients/pigments present in the sample if any. In such case alternative validated chromatographic conditions are acceptable in order to improve/achieve clear separation, or reproducibility. Responsibility of changes in chromatographic conditions lies with manufacturer.

ANNEX F

[*Table* 1, *Sl No.* (iv)]

DETERMINATION OF ABSENCE PICRAMIC ACID

F-1 APPARATUS

F-1.1 Volumetric Flasks, 10 ml

F-1.2 Analytical Balance

F-1.3 Development Chamber or Beaker, spoutless

F-1.4 Glass Rods

F-1.5 Pipette (1 × 10 ml)

F-1.6 TLC Plates (Pre-coated silica gel 60 $\mathrm{F_{254}}$, 0.2 mm thickness)

F-1.7 Silicone Wax

F-1.8 Syringe, 5 or 10 µl capacity

F-2 REAGENTS AND CHEMICALS

F-2.1 Acetonitrile

F-2.2 Methanol

F-2.3 Glacial Acetic Acid

F-2.4 Lawsone (reference standard)

F-2.5 Picramic Acid (reference standard)

F-3 PREPARATION OF PLATES

Mix 10 g of the silica gel (of TLC grade, particle size 10 to 40 µm) with 20 ml of distilled water to make a slurry and spread over glass plates to a depth of 250 µ. Activate the plates for 30 min, by keeping in an oven maintained at 105°C.

NOTE — Precoated silica gel 60 $\mathrm{F_{254}}$ plate may also be used.

F-4 PREPARATION OF LAWSONE REFERENCE SAMPLE

Dissolve about 10 mg of lawsone reference sample with 10 ml of chloroform and apply one drop of the extract on the base line of the plate.

F-5 PREPARATION OF PICRAMIC ACID REFERENCE SAMPLE

Dissolve about 10 mg of picramic acid reference sample with 10 ml of chloroform and apply one drop of the extract on the base line of the plate.

F-6 PREPARATION OF HENNA POWDER REFERENCE SAMPLE

Extract[1] about 1.0 g of the henna (*MEHENDI*) powder reference sample previously prepared from authentic henna leaves with 10 ml of chloroform and apply 5 µl to 10 µl of the extract on the base line of the plate.

F-7 PREPARATION OF SAMPLE

Extract[1] about 1.0 g of the prepared sample with 10 ml of chloroform and apply 5 µl to 10 µl of the extract on the base line of the plate.

F-8 DEVELOPMENT OF CHROMATOGRAM

Keep the prepared plates in a jar containing a mixture of acetonitrile : methanol : glacial acetic acid (9 : 1 : 0.05). Observe the spots obtained as such and under ultra violet light.

Elution order (lowest R_f to highest R_f) — lawsone, picramic acid, chlorophyll

F-9 OBSERVATION

Compare the TLC pattern due to sample solution with that of lawsone reference sample, picramic acid and henna (*MEHENDI*) powder reference sample which is prepared from authentic henna (*MEHENDI*) leaves.

Check for spot corresponding to picramic acid in colour and characteristics for the sample on the plate. This indicates the presence of picramic acid.

NOTE — Confirmatory test may be necessary to conclude the presence of picramic acid. This may be done by scraping out the respective spot and solubilizing and checking using other analytical methods like spectrophotometric, HPLC etc.

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[1] More time may be required to ensure appropriate extraction of lawsone from the leaf powder.

ANNEX G

[*Table* 1, *Sl No.* (v)]

DETERMINATION OF HEAVY METALS

G-1 OUTLINE OF THE METHOD

The color produced with hydrogen sulphide solution is matched against that obtained with standard lead solution.

G-2 APPARATUS

G-2.1 Nessler Cylinders — 50 ml capacity.

G-3 REAGENTS

G-3.1 Dilute Hydrochloric Acid — Approximately 5 N.

G-3.2 Dilute Acetic Acid — Approximately 1 N.

G-3.3 Dilute Ammonium Hydroxide — Approximately 5 N.

G-3.4 Hydrogen Sulphide Solution — Standard.

G-3.5 Standard Lead Solution — Dissolve 1.600 g of lead nitrate in water and make up the solution to 1 000 ml. Pipette out 10 ml of the solution and dilute again to 1 000 ml with water. One milliliter of this solution contain 0.01 mg of lead (as Pb).

G-4 PROCEDURE

Weigh accurately about 2.000 g of material in crucible and heat on a hot plate and then in a muffle furnace to ignite it at 600°C to constant mass. Add 3 ml of dilute hydrochloric acid, warm (wait till no more dissolution occurs) and make up the volume to 100 ml. Filter the solution. Transfer 25 ml of the filtrate into a Nessler's cylinder. In the second Nessler's cylinder, add 2 ml of dilute acetic acid, 1.0 ml of standard lead solution and make up the volume with water to 25 ml.

Add 10 ml of hydrogen sulphide solution to each Nessler cylinder and make up the volume with water to 50 ml. Mix and allow to stand for 10 min. Compare the colour produced in the two Nessler's cylinders. Blank determination without samples are recommended to avoid errors arising out of reagents.

G-5 RESULTS

The sample may be taken to have passed the test, if the colour developed in the sample solution is less than that of standard solution.

ANNEX H

[*Table* 1, *Sl No.* (vi)]

DETERMINATION OF ARSENIC

H-1 OUTLINE OF THE METHOD

Arsenic present in a solution of the material is reduced to arsine, which is made to react with mercuric bromide paper. The stain produced is compared with a standard stain.

H-2 REAGENTS

H-2.1 Mixed Acid — Dilute one volume of concentrated sulphuric acid with four volumes of water. Add 10 g of sodium chloride for each 100 ml of the solution.

H-2.2 Ferric Ammonium Sulphate Solution — Dissolve 64 g of ferric ammonium sulphate in water containing 10 ml of mixed acid and make up to one liter.

H-2.3 Concentrated Hydrochloric Acid [*see* IS 265 : 1993 Hydrochloric acid — Specification (*fourth revision*)]

H-2.4 Stannous Chloride Solution — Dissolve 80 g of stannous chloride ($SnCl_2.2H_2O$) in 100 ml of water containing 5 ml of concentrated hydrochloric acid.

H-3 PROCEDURE

Carry out the test as prescribed in IS 2088, adding into the Gutzeit bottle, 2 ml of ferric ammonium sulphate solution, 0.5 ml of stannous chloride solution and 25 ml of sample solution as prepared in **G-4.**

For comparison, prepare a stain using 0.001 mg of arsenic trioxide.

Bureau of Indian Standards

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Amendments are issued to standards as the need arises on the basis of comments. Standards are also reviewed periodically; a standard along with amendments is reaffirmed when such review indicates that no changes are needed; if the review indicates that changes are needed, it is taken up for revision. Users of Indian Standards should ascertain that they are in possession of the latest amendments or edition by referring to the latest issue of 'BIS Catalogue' and 'Standards: Monthly Additions'.

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